August 13, 2013

THE ALS ASSOCIATION’S GLOBAL RESEARCH AWARDS 2013
The ALS Association’s latest research awards include funding commitments of $4.3 million to scientists in laboratories in 14 states in the United States as well as in the United Kingdom, Ireland, Belgium, Canada, Argentina, France and Italy. These awards, which support 35 new projects, are part of its Translational Research Advancing Therapies for ALS (TREAT ALS™) portfolio, a diverse portfolio of amyotrophic lateral sclerosis (ALS) research to find treatments and a cure for Lou Gehrig’s Disease. These new awards include Investigator-Initiated Awards, The ALS Association-Initiated Awards and the Milton Safenowitz Post-Doctoral Fellowship for ALS Research Awards.

Donations from the following organizations and individuals enabled The ALS Association to fund these new research grants: Greater Philadelphia Chapter, Greater New York Chapter, The Jeff Kaufman Fund of the Wisconsin Chapter, Greater Chicago Chapter, Texas Chapter, Golden West Chapter, Greater Sacramento Chapter, Orange County Chapter, Greater San Diego Chapter; The Motor Neuron Disease Association of the United Kingdom; and Jay and Toshiko Tompkins.

ALS Association chapters provide ongoing and generous support to fund these new research grants. Chapters continually work with certified centers and clinics to provide the best care to people living with ALS.

INVESTIGATOR-INITIATED AWARDS
Investigators submit a proposed line of inquiry covering diverse areas of research, and The ALS Association convenes a scientific review committee to discuss proposals and make selections based on the merits of each. There are two types of Investigator-Initiated Awards: innovative discovery awards and multi-year awards.

Researcher: Osvaldo Uchitel, M.D., Ph.D., Universidad De Buenos Aires, Argentina

Topic: Identification of Candidate IgG Biomarkers for ALS via Combinatorial Library Screening

Description: A biomarker, like a specific antibody, is a substance used as an indicator of a normal or pathogenic processes, or pharmacologic responses to a therapeutic intervention. The presence of antibodies in ALS patients but not in healthy patients may indicate an immune-mediated mechanism in motor neuron degeneration. The pathophysiological relevance of
autoantibodies (self-reactive antibodies) in ALS remains controversial mainly because it was not possible to identify the antigen(s) responsible for the autoimmune response. Recently, a novel approach to identify disease-related antibodies was designed using peptoid molecules (small amino acid chains) to build a combinatorial library. Thousands of peptoids with different molecular shapes mimic some aspects of the three-dimensional characteristics of the (unknown) native antigen(s) recognized by disease-specific antibodies and thus serve as relatively high-affinity capture agents. Those molecules that retain far more antibodies from the case samples compared to the controls are identified and subsequently tested as capture agents for diagnostically useful antibodies. The utility of this method was already demonstrated using a mouse model for multiple sclerosis and via the identification of IgG biomarkers for Alzheimer’s disease.

**Researcher:** Agnes Nishimura, M.D., Ph.D., Institute of Psychiatry, Kings College, London, United Kingdom

**Topic:** Modeling ALS Using Induced Pluripotent Stem Cells Generated by TALEN Technology

**Description:** Amyotrophic lateral sclerosis (ALS) in most people is caused by unknown factors; however, about 10 percent of people have a family history of ALS or a family history of frontotemporal dementia. Whatever the underlying cause of ALS, the major pathological change is the accumulation of the TDP-43 protein in affected neurons in the brain and spinal cord. Around 50 different mutations have been identified in the gene for TDP-43 accounting for 1-4 percent of families. This confirms a central role for TDP-43 in directly causing motor neuron degeneration. These particular mutations were found in ALS patients as opposed to healthy individuals, suggesting their causal effect. In the past few years the development of new techniques has greatly helped to understand the mechanisms by which these mutations may be causing the disease. One such technique consists in transforming skin cells into stem cells. These stem cells are capable of becoming any type of cell from the body under certain experimental conditions. They can be transformed into motor neurons, which die in ALS. The researchers have successfully generated stem cells from skin cells collected from patients with ALS and have shown that a few characteristics of these stem cells are also observed in the brain cells from these patients. In this research, they aim to generate new cell models for ALS by introducing known mutations into cells, which have successfully been turned into stem cells. In addition, they will replace the mutation with the normal version of DNA as found in healthy individuals and compare both cell lines. The generation of these novel lines has the potential to broaden the understanding of ALS pathology and lead to new targets for drug screening of therapeutic compounds for ALS.

**Researcher:** Elke Bogaert, Ph.D., K U Leuven, Belgium

**Topic:** Identification of Disease Modifying Genes in ALS-FUS Small Animal Models

**Description:** Mutations in Fused in Sarcoma/translocated in liposarcoma, FUS/TLS, have been described as a genetic cause of ALS. The precise mechanism by which these mutations cause disease is unknown. The researchers have created two small animal models, a fruit fly
and a zebrafish model, to study the mechanisms by which these proteins alter normal functioning of motor neurons and to identify potential therapeutic targets. To do so, they will screen the complete genome of the fruit fly to find potential genes able to modify the defects observed in the fly. Potential candidate genes will be further investigated in our zebrafish model. They have chosen the two small animal models, as they have a fast generation time and a whole genetic toolbox available, which makes it possible to screen the entire genome within a reasonable amount of time. Ultimately they hope that the identified genes can reveal novel potential therapeutic targets. Not only mutation in FUS but also in TDP-43 and in C9orf72 is thought to alter proper RNA metabolism in motor neurons, and therefore they hope these results will be translatable to the whole ALS spectrum.

**Researcher:**  Fen Biao Gao, Ph.D., University of Massachusetts Medical School, Worcester, Massachusetts

**Topic:** Use of Patient-Specific iPS Cells to Investigate Pathogenic Mechanisms of Hexanucleotide Repeats in C9orf72

*Funded by The ALS Association, Greater Chicago Chapter, through the State of Illinois*

**Description:** Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig’s Disease, causes paralysis due to motor neuron degeneration. Unfortunately, there is no effective treatment. The recent breakthrough in induced pluripotent stem cell (iPSC) technology allows scientists to generate an unlimited number of human motor neurons that harbor endogenous genetic mutations found in ALS patients for studies of pathogenic mechanisms in vitro. In this project, the researchers will generate more iPSC lines and also take advantage of iPSCs they already generated from patients with over 1,000 copies of GGGGCC repeat expansions to understand how this mutation causes the disease. Specifically, they will further characterize RNA foci and RAN translation (the source of abnormal peptides in C9orf72 mutant cells) and examine their toxicity in human neurons derived from iPSC lines containing GGGGCC repeats. Moreover, they will test the ability of antisense oligonucleotides (ASOs) and siRNAs to reduce the number of repeat-containing RNA foci and/or RAN translation products in human neurons derived from patient-specific iPSC lines. Because the abnormal repeat expansion is the most common genetic mutation in ALS, the proposed studies represent a powerful new avenue for identifying potential therapeutic targets.

**Researcher:**  Paola Arlotta, Ph.D., Harvard University, Cambridge, Massachusetts

**Topic:** Directed Differentiation of Upper Motor Neurons From Human iPS Cells for Disease Modeling of Amyotrophic Lateral Sclerosis

**Description:** Amyotrophic lateral sclerosis is characterized by the selective degeneration of two classes of neurons: those that connect the brain to the spinal cord (upper motor neurons) and those that connect the spinal cord to the skeletal muscles (lower motor neurons). The consequent progressive paralysis is typically fatal within 3-5 years. Here, the researchers aim to direct the generation of upper motor neurons from both healthy and ALS patient-specific iPSC
cells. The work aims to mimic the upper motor neuron pathology of ALS in vitro to both understand disease pathology and to enable drug screening.

**Researcher: Stefania Corti, M.D., Ph.D., University of Milan, Italy**

**Topic:** Amyotrophic Lateral Sclerosis (ALS) Therapy via Minimally Invasive Transplantation of a LeX+CXCR4+VLA4+ Neural Stem Cell Subpopulation Derived from Induced Pluripotent Stem Cells

**Description:** Amyotrophic lateral sclerosis (ALS) is a fatal neuromuscular disease with a tremendous burden on the patients and their families as well as a significant impact on society and public health. Finding effective therapeutic strategies is crucial for ALS patients since currently no treatment exists. Increasing attention has been given during the last years to stem cell therapy as a promising treatment for neurodegenerative disorders. Neural stem cells (NSCs) could play a relevant therapeutic role through a number of different actions, including production of trophic factors, neuroprotection, and preservation of neuromuscular function. The success of a cellular approach can be achieved by increasing the understanding of stem cells features and identification of variables that play a major role in the success of transplantation. The researchers’ aim is to develop a safe, effective, minimally invasive NSC transplantation protocol in ALS animal models, testing a specific subset of NSCs (LewisX+CXCR4+VLA4+) in order to improve NSC migratory capacity and efficacy. Indeed these cells will be genetically modified to increase their engraftment and therapeutic potential. This work can pave the way for the clinical translation of cell-mediated therapy in ALS patients, as well as in the treatment of other neurodegenerative diseases.

**Researcher: Hande Ozdinler, Ph.D., Northwestern University, Chicago, Illinois**

**Topic:** Molecular Mechanisms That Underlie Corticospinal Motor Neuron Vulnerability and Degeneration in ALS

* Funded by The ALS Association, Greater Chicago Chapter, through the State of Illinois

**Description:** Different from other motor neuron diseases, ALS is characterized by the progressive degeneration of both the cortical and spinal motor neurons. Therefore, mechanisms that are involved in upper motor neuron degeneration must be studied together with spinal motor neurons. This proposal involves the use of upper motor neurons in an effort to expand the understanding of the selective motor neuron vulnerability in neurodegenerative diseases and therefore has a unique importance and great relevance to ALS. There have been important advances in the understanding of cellular and molecular mechanisms that lead to spinal motor neuron degeneration, but the factors that are involved in upper motor neuron vulnerability remain unknown. The Ozdinler Laboratory has generated novel tools and approaches in an effort to shed light onto the upper motor biology in ALS. Upon completion of this proposal, they will have an understanding of the molecular and genetic mechanisms that are selectively upregulated and downregulated in vulnerable motor neurons. These findings will reveal the pathways that are selectively activated/inactivated and will point to the presence of potential
early detection markers in ALS. This research is relevant to ALS and the findings will be crucial for developing effective and long-term treatment strategies.

**Researchers: Luc Dupuis, Ph.D., Inserm, France**  
**Clotilde Lagier-Tourenne, Ph.D. MD. University of California, San Diego**

**Topic:** Understanding the Genetic Mechanisms of FUS-Mediated ALS Through a Conditional Knock-In Mouse Model

**Description:** Mutations in the FUS gene are responsible for a subset of familial ALS cases with young onset and very rapid disease progression. The protein encoded by the FUS gene accumulates in the cytoplasm of neurons in patients with FUS mutations and also in many patients with sporadic forms of the disease. The researchers recently generated a mouse model with a targeted mutation in the Fus gene that leads to the truncation of the FUS protein and should lead to its cytoplasmic accumulation, thereby closely mimicking what is happening in patients. Mice with two copies of this mutant gene die at birth from spinal muscular atrophy, a disease closely related to ALS. The objective of this project is to determine whether mice with one copy of the mutant gene develop symptoms of ALS and to understand whether FUS mutations lead to ALS through a loss of the function of FUS protein or through the gain of a novel adverse property. This project will greatly increase the knowledge on the pathophysiological function of FUS in ALS and hopefully provide a novel animal model of the disease.

**Researcher:** Tom Maniatis, Ph.D., Columbia University, New York, New York

**Topic:** Understanding ALS Disease Mechanisms Using Human TDP-43 iPS-derived Motor Neurons  
*Funded by The ALS Association, Greater New York Chapter*

**Description:** The use of human induced pluripotent stem cells (iPS cells) to study ALS disease mechanisms in iPS-derived motor neurons (iMNs) has generated considerable excitement. However, a robust pathological phenotype comparable to that reported for mouse ES cell-derived motor neurons has not been observed with ALS-iMNs, which display only subtle phenotypes. One of the primary differences between the mouse and human studies may be the difference in time required for mouse and human motor neurons to mature in culture. Studies in the Maniatis lab have focused on iPS cells generated from patient fibroblasts bearing the TDP43 M337V mutation. They have extended and modified the protocol developed for the mouse ES model in which iMNs are cultured over primary astrocytes (sandwich cultures) for over four weeks, a period long enough for iMNs to mature in vitro. Characterization of these long-term iMNs in culture reveals that these cells express a variety of motor neuron markers that are not observed at the early times in culture. Based on these observations, they are thoroughly characterizing iMNs from both control and TDP43 mutant iPS cells. Their goal is to understand the mechanisms by which TDP43 mutant iMNs display a differential response to mutant astrocytes in their “physiological toxicity” model.
Researcher: Katrin Andreasson, M.D., Stanford University, Palo Alto, California

Topic: Targeting Toxic Protein Adduction by Reactive Aldehydes in ALS

Description: Oxidative stress contributes significantly to progression of ALS and occurs because of abnormally elevated free radical generation in the central nervous system of ALS patients. Oxidative stress results in biological toxicity through the oxidation of cellular lipids and the generation of a class of uniquely toxic and highly reactive lipid aldehydes. Because of their extreme reactivity, these lipid aldehydes rapidly and irreversibly bind to a broad range of proteins within the cell, and in doing so disrupt normal protein functions, leading to cellular injury and cell death. Thus, reactive lipid aldehydes exert significant, broad spectrum and immediate biological toxicity that likely accelerate disease progression in ALS. The researchers propose to use a brain penetrant small molecule to "scavenge" these reactive aldehydes and prevent them from binding to proteins and disrupting vital protein functions. They aim to show proof-of-concept that targeting these uniquely reactive aldehydes can reduce or halt disease progression in two mouse genetic models of ALS. Successful completion of the proposed research will identify highly reactive aldehydes as a new therapeutic target in ALS and introduce a novel approach using brain penetrant small molecules that could be rapidly moved into clinical trials for patients with ALS.

Researcher: Robert Reenan, Ph.D., Brown University, Providence, Rhode Island

Topic: Genetic Suppression Studies of Human SOD1-Based ALS Mutations in a Drosophila Model

Description: One common cause of familial ALS was discovered more than two decades ago, namely, mutations in Superoxide Dismutase 1 gene. Despite this advance knowledge, there are no therapies that dramatically alter the outcomes for a patient diagnosed with ALS. The researchers have developed a model of ALS in the genetically tractable system, Drosophila melanogaster (fruit fly). Introducing human disease-causing mutations into the fly genome, they generate models that appear to recapitulate the most important aspects of ALS, including the lethal loss of motor control. Preliminary data support the notion that flies lose, or lack function, of motor neurons. In this grant, they will further characterize their models of ALS (including genomic methods) to identify biomarkers associated with this model of ALS. Then, they will propose to utilize the power of Drosophila forward genetics to mutate the genomes of affected flies, looking for secondary mutations in other genes that reverse lethality of ALS mutations. In effect, they will identify conserved pathways and processes that “cure” affected flies and point to new avenues of intervention. Because this approach is a priori unbiased, it will accept any mutations that rescue the flies, most importantly, those that have not been pursued based on current models of ALS.

Researcher: Jill Zitzewitz, Ph.D., University of Massachusetts Medical School, Worcester, Massachusetts
Topic: Developing Small Molecule Screening Assays for Identifying Potential Therapeutics to Treat ALS
*Funded by The Jeff Kaufman Fund of the The ALS Association, Wisconsin Chapter

Description: Misfolding and aggregation of proteins is responsible for a wide host of human diseases including ALS. The researchers' lab has shown that mutations at dozens of locations in Cu, Zn superoxide dismutase 1 (SOD1) result in increased populations of partially folded forms that may play a role in aggregation and can be targets for therapeutic interventions. A recent screen for small molecules to stabilize the SOD1 protein yielded several promising potential therapeutics for ALS. However, because mutations in SOD1 only affect about 2 percent of all ALS cases, there is a critical need to devise therapeutics for the 90 percent of cases resulting from unknown genetic cause (sporadic ALS). A majority of patients with sporadic ALS have protein aggregates composed of TDP-43, and drug screens that target TDP-43 may prove beneficial to prevent misfolding, aggregation and toxicity. The researchers' results on TDP-43 indicate the presence of a partially-folded state that may act as a molecular hazard. They propose to develop assays to screen for small molecules that stabilize TDP-43 to inhibit its tendency to form aggregates and to prevent toxicity. These studies will provide the framework for the design and development of novel therapeutics for this devastating disease.

Researcher: Roel Ophoff, Ph.D., University of California at Los Angeles

Description: Discoveries in the last few years have highlighted that RNA processing may play an important role in ALS pathogenesis. For example, mutations in genes encoding RNA binding proteins TDP-43 and FUS/TLS cause familial ALS. However, the vast majority (>90 percent) of ALS patients have no family history of the disease. The question is whether the same molecular mechanisms observed in familial ALS also play a role in sporadic patients. The researchers' study is aimed to investigate the genetic link between RNA processing and sporadic ALS. They will study genetic variation in TDP-43 and FUS/TLS and their gene targets. In a sample of 250 ALS patients (90 percent sporadic), they will identify all rare and common sequence variations in these target genes that may affect binding to TDP-43 and FUS/TLS, followed by examining these variants in large cohorts of >3,000 patients. Moreover, they will study transcriptome differences in patients with and without sequence variants in these gene targets. While high-throughput sequencing of genomic DNA will give insight into genetic mechanisms of disease susceptibility, RNA sequence data will provide immediate insight into functional effects of these variants in RNA processing and splicing. Positive results may lead to new drug or treatment targets for ALS.
Researcher:  Lawrence Hayward, M.D., Ph.D., University of Massachusetts Medical School, Worcester, Massachusetts

Topic:  Mouse Models of FUS/TLS Expression in Amyotrophic Lateral Sclerosis

Description:  Recent genetic discoveries implicate mutant proteins involved in RNA processing in the pathogenesis of amyotrophic lateral sclerosis (ALS), and there is an urgent need to characterize informative animal and cellular models based on these clues. One such gene encodes a key nucleic acid binding protein (FUS/TLS) that is broadly distributed throughout the body but is particularly abundant in the motor neurons that die prematurely in ALS. The researchers propose to investigate the specific mechanisms by which mutant variants of human FUS/TLS expressed in mice disrupt the normal function or survival of motor neurons and supporting cells in the nervous system. They will test whether motor neurons are preferentially vulnerable to loss of the FUS/TLS protein and whether the FUS/TLS mutants are deficient in one or more of its normal activities. They will also ask whether the FUS/TLS mutants perturb critical functions within the control center of the cell (the nucleus) that could explain their toxicity. In addition, they will use a novel technology to edit the mouse genome to introduce changes corresponding to the human mutations into the mouse FUS/TLS gene. Insights obtained from these models will accelerate the progress toward identifying novel targets for the treatment of ALS.

Researcher:  David Corey, Ph.D., University of Texas Southwestern, Dallas

Topic:  Disease Process of ALS, Therapies for ALS Cell Targets, RNA Therapy

*Funded by The ALS Association, Texas Chapter

Description:  Neurological, neuromuscular and neurodegenerative disorders can all arise from the expansion of simple nucleotide repeat sequences in the genome. Their expression as RNA molecules that contain these repeats is proposed to contribute to disease by disrupting RNA-binding protein function and normal gene expression programs. A new disease having features of either frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS), or both, is now included in this group of repeat expansion diseases. This new disease, called c9FTD/ALS, is caused by a simple repeat expansion in an intron of the gene C9ORF72. c9FTD/ALS is the most common form of inherited ALS and FTD. The eventual treatment of c9FTD/ALS lies in understanding the contribution of non-coding repeat expansion RNA to the molecular pathology of disease. It also relies on development of potential therapeutics as well as simple benchmarks for gauging the efficacy of those therapeutics. The researchers will address these shortfalls by developing cell-based models of c9FTD/ALS disease, discovering new disease mechanisms, establishing benchmark assays for therapeutic testing, and testing small nucleic acids as preliminary therapeutic candidates. These results will help the research community move toward therapeutic approaches for c9FTD/ALS and potentially other neurological disorders that share similar molecular pathology.

Researcher:  Jean Pierre Julien, Ph.D., Laval University, Quebec City, Canada

Topic:  Therapeutic Effects of Ashwagandha in Two Mouse Models of ALS
Description: The researchers have found that Withaferin A, an active compound present in herbal medicine *Withania somnifera* (Ashwagandha), conferred therapeutic effects in several mouse models of ALS. *Withania somnifera* has been used in Indian medicine for 3,000 years for treatment of rheumatoid arthritis, asthma, inflammatory bowel disease and cancer. The compound is easily accessible and was well tolerated in previous studies. Considering the robust therapeutic effects of Withaferin A in different ALS mice, they propose to test the therapeutic effects of Ashwagandha administered orally in two mouse models of ALS, the SOD1G93A mice and transgenic mice expressing genomic fragment of TDP-43 A315T, which develop pathological hallmarks of human ALS such as TDP-43 aggregates.

**Researcher:** Ludo Van Den Bosch, Ph.D., KU Leuven and VIB, Belgium

**Topic:** Role of Histone Deacetylase 6 (HDAC6) in Amyotrophic Lateral Sclerosis

Description: Axonal transport defects play an important role in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS). The acetylation level of Alpha-tubulin, one of the building blocks of the microtubules, seems to play a crucial role in the regulation of axonal transport. The researchers deleted HDAC6, the major alpha-tubulin deacetylating enzyme, in a mouse model of ALS and discovered that the disease onset was unaltered but that survival was significantly increased. In this project, they will confirm the potential role of HDAC6 expression by determining whether selective inhibition of HDAC6 has an effect on disease onset and survival.

**Researcher:** Bradley Foerster, M.D., University of Michigan, Ann Arbor

**Topic:** Translating Novel Positron Emission Tomography Biomarkers for ALS

Description: There is increasing evidence that inflammation pays a key role in the disease process of ALS. Inflammatory changes may be present before the significant loss of neurons in ALS and may serve a target for earlier diagnosis and treatments. A key barrier to further understand the impact of inflammation in ALS is the lack of a test to measure the degree of inflammation in the nervous system. A recently developed positron emission tomography (PET) imaging agent shows significant potential in targeting the inflammatory process in the central nervous system. This study will use this new PET imaging agent to explore the inflammation process in ALS. The goal is to use this new PET agent as a diagnostic and treatment-monitoring test.

**Researcher:** Bruce Trapp, Ph.D., Cleveland Clinic, Cleveland, Ohio

**Topic:** Postmortem MRI and Rapid Autopsy of ALS Patients to Reveal Pathogenic Targets and Validate MRI Metrics of Motor Neuron and Associated Microenvironment Pathology

Description: A major obstacle in the development of therapies for ALS is that the disease mechanism is unknown. Extensive research using mouse models supports the hypothesis that ALS is a disease that involves other cell types besides the motor neurons, and that abnormalities in cells that are in the neighborhood of motor neurons have a deleterious effect on their function. In this project we will set-up a magnetic resonance imaging (MRI)/rapid autopsy
program for ALS patients in which postmortem MRI will be performed immediately after death followed by autopsy of the brain and spinal cord. Histopathological analyses of the brain and spinal cord tissues will be performed to identify the role of the neighboring cells on motor neuron disease. We will then translate these findings to the clinical setting and enable them to be used in living patients by correlating our histopathological results with the postmortem MRI, yielding validated MRI signatures of the different types of pathology we observed in the motor neurons and their neighboring cells.

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Researcher:  Timothy Miller, M.D., Ph.D., Washington University, Saint Louis, Missouri
Topic:  Determining the Half-life of SOD1 in Human CSF
* Funded by the Greater Philadelphia Chapter of The ALS Association

Description:  Dr. Miller’s team has developed a potential therapy (antisense oligonucleotides) for patients with ALS due to mutations in the gene superoxide dismutase 1 (SOD1). The goal of this therapy is to lower levels of the protein SOD1. They have recently completed a Phase I safety trial using these antisense oligonucleotides and have confirmed that the therapy is safe. For the next trial, Phase II, the most important read-out for the therapy will be the ability to decrease SOD1 in the cerebral spinal fluid (CSF), the fluid that bathes the brain and the spinal cord and is easily sampled via lumbar puncture. A key question is how long after starting the antisense oligonucleotide therapy should the SOD1 levels be expected to decrease in the CSF, which will determine when the lumbar puncture is performed in subjects. To begin to answer that question, the investigators need to know how quickly the SOD1 normally is degraded. They will complete a small human study to figure out how quickly SOD1 is normally degraded in the CSF. This will be a key piece of data for the Phase II trial.

Researcher:  Murray Grossman, M.D., University of Pennsylvania, Philadelphia
Topic:  Genetic Status of Asymptomatic ALS

Description:  While amyotrophic lateral sclerosis (ALS) was thought to be free of cognitive impairment, recent work shows that 33-50% of patients have cognitive deficits and 10% have frontotemporal lobar degeneration (FTLD). The protein TDP-43 forms aggregates in the brain and spinal cords in most ALS patients, and the same pathology is also found in 50% of FTLD patients, linking ALS and FTLD. 10% of ALS is familial (fALS), and TDP-43 pathology is frequently found in fALS. Another link between ALS and FTLD recently emerged with identification of C9orf72 expansion, where families include both ALS and FTLD. 10% of C9orf72 carriers are sporadic. The researchers will recruit families with a C9orf72 expansion or other mutations, or with a strong family history, and compare asymptomatic carriers with asymptomatic family members who are not carriers. They will collect biofluid, and perform
neuropsychological assessments and multi-modal MRI. They will next implement two novel, cost-effective genetic screens in these cases. One panel genotypes 46 risk alleles derived from genome-wide association studies to identify carriers of mutations. The second is a targeted next-generation sequencing panel which includes coding regions of 45 genes associated with ALS, FTLD, and related conditions. Their intent is to cost-effectively identify C9orf72 expansion and other mutations in asymptomatic family members and symptomatic individuals with ALS/FTLD spectrum disorders.

Researcher: Zuoshang Xu, M.D., Ph.D., University of Massachusetts Medical School, Worcester, Massachusetts

Topic: AAV Therapeutic Agents for ALS

Description: Gene therapy is potentially a highly effective means to deliver therapy for diseases of the central nervous system, including ALS. A major challenge for applying gene therapy to treat ALS, a disease that affects broad areas of the CNS including the cortex, brainstem and the entire length of the spinal cord, is the difficulty in spreading the virus to all the areas that are affected by the disease. In this project, the researchers will develop rAAVrh.10 vector for treatment of ALS. They will test this vector by using it to deliver RNAi against mutant SOD1 in a transgenic rat. If successful, these experiments will provide data to support the further development and clinical trial of this therapeutic strategy.

Researchers: Kevin Boylan, M.D., and Leonard Petrucelli, Ph.D., Mayo Clinic in Jacksonville, Florida

Topic: Discovery and Validation of ALS Biomarkers

Description: Recently, it was discovered that a mutation, specifically a longer than usual repeating sequence (GGGGCC) in the C9ORF72 gene leads to the development of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). While the exact process through which this mutation causes disease is not yet known, the researchers have found that it leads to the expression of abnormal peptides, the building blocks of proteins, within neurons that would otherwise not exist. They identified these peptides through the generation of novel antibodies that bind the peptides and serve as a marker of their presence. Because these peptides are present only in ALS and FTD patients with the C9ORF72 mutation, the researchers believe they can serve as a biomarker for this population of patients. To investigate this, they will develop new antibody-based immunoassays to measure levels of the abnormal peptides in cerebrospinal fluid and blood of ALS patients with or without the C9ORF72 mutation. They will then determine whether levels of abnormal peptides in these biofluids are indicative of the presence of the C9ORF72 mutation or correlate with neuropsychological and motor symptoms. Such immunoassays, or screening tests, may establish a basis for the diagnosis of patients with the C9ORF72 mutation and for monitoring the progression of disease in affected individuals.

Researcher: Peter Bauer, M.D., Ph.D., Mayo Clinic in Jacksonville, Florida
Role of C9ORF72 Protein in C9-associated ALS/FTD: iPSC-derived neuronal modeling with genome editing using TALEN technology

Amyotrophic lateral sclerosis (ALS) is a rapidly progressing disease that affects motor neurons in the brain and spinal cord. The cause of 90-95 percent of ALS cases is unknown, while approximately 5-10 percent of ALS cases are due to inherited genetic causes. Mutations in the C9ORF72 gene have recently been established as the most common genetic cause of ALS and frontotemporal lobar degeneration (FTLD), a disorder that is one of the major causes of dementia in adults younger than 65 and includes abnormalities in behavior, language and personality. In patients with C9ORF72 mutations, lower levels of C9ORF72 transcripts were detected in the brain and may lead to lower levels of C9ORF72 protein. Whether decreased levels of C9ORF72 protein directly cause disease is unknown, in part because it remains unclear what are the functions of C9ORF72 in the brain. New research focuses on investigating either how repeat expansions in the C9ORF72 gene affect RNA levels and the level of peptides produced from this RNA by unconventional translation or elucidating the function of the C9ORF72 protein. Given that the information from both approaches may provide greater insight into many neurodegenerative diseases, this project aims to unite the two research concepts in order to gain a better understanding of C9ORF72 function in the presence or absence of pathogenic GGGGCC expansion in the C9ORF72 gene and to determine whether loss of C9ORF72 causes neuronal death. For this purpose, the researchers will produce neurons from induced pluripotent stem cells (iPSC) generated from skin fibroblasts obtained from patients with C9ORF72 mutations and from controls. They will artificially modulate the levels of endogenous C9ORF72 protein by genomic modification. Moreover, they will generate partial deletion mutants to identify domains playing roles in the disease process and neuronal viability.

Richard Barohn, M.D., University of Kansas in Kansas City

Phase 2 Study of Rasagiline for Treatment of ALS

Rasagiline is approved for the treatment of Parkinson's disease. In addition, rasagiline may be an effective treatment for other neurologic diseases. Considering that mitochondrial function is altered in both Parkinson's disease and ALS, it is reasonable to consider that rasagiline could benefit patients with ALS. It has already been demonstrated that rasagiline prolongs survival in the mouse model of ALS. In one small clinic experience from Israel, rasagiline treatment was associated with slower deterioration in ALS patients. The researchers are nearing completion of a 36-patient, 12-month, open-label study of rasagiline and have done preliminary work with blood biomarkers to measure mitochondria function. They are now beginning an 80-patient, placebo-controlled rasagiline study. In this new study, they have refined their measurement of blood and urine biomarkers of mitochondrial function, oxidative stress and apoptosis to determine if there is target engagement by rasagiline. They have also included brain MRIs at KUMC to study oxidative stress. If they show either an indication of disease slowing or target engagement with rasagiline, this will lay the groundwork for a larger efficacy treatment trial of rasagiline in ALS.

John Landers, Ph.D., University of Massachusetts Medical School, Worcester, Massachusetts
Description: The etiology of sporadic ALS (SALS), which constitutes 90 percent of all cases, is largely unknown, but genetic factors likely play a major role in susceptibility to the disease. Previous studies to identify these genetic factors have been based on the hypothesis that the risk is influenced by common, weakly associated alleles. These studies have failed to produce robustly replicated results. On the contrary, several lines of evidence suggest that rare variants may be responsible for a large part of SALS heritability. The recent development of novel sequencing technologies now allows screening of cohorts for rare coding variants at a genome-wide scale. Here, the researchers propose to sequence the coding region of 200 SALS cases in an effort to identify rare variants associated with susceptibility to SALS. Their study is designed to exploit selective sampling of SALS patients with an early disease onset, indicative of a strong genetic component, and to adopt complementary methods for statistical analysis in order to maximize the chances of success. Furthermore, to enhance ALS research in the scientific community, they will establish a public database of their SALS sequencing results. This database will reduce the time and expense for the scientific community to identify additional genes contributing to ALS.

**Researcher:** Laura Ranum, Ph.D., University of Florida, Gainesville

Topic: Development of a Public Searchable Variant Database for ALS Exomes

* Funded by The Jeff Kaufman Fund of the The ALS Association, Wisconsin Chapter and The Motor Neuron Disease Association in the United Kingdom

Description: A novel form of amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD) was recently shown to be caused by a DNA mutation in which six letters of the genetic code, “GGGGCC”, are repeated extra times. This discovery links ALS/FTD with a large group of other neurological diseases caused by similar repeat expansion mutations and is particularly exciting because lessons learned during the past 20 years from studying these other diseases are likely to be relevant to the more common ALS/FTD. The Ranum laboratory has followed a family with ALS for more than 20 years and recently discovered that this family has the GGGGCC expansion mutation. They have cloned the repeat expansion mutation from a member of this family and propose a series of experiments, including the generation of a mouse model to better understand how this expansion mutation causes disease.

**Researchers:** Janice Robertson, Ph.D. University of Toronto, Toronto, Canada
Joan Coates, University of Missouri, Missouri

Topic: Molecular Genetics of the G4C2 expansion mutation in ALS/FTD

An active immunization strategy targeting monomer-misfolded SOD1 has shown efficacy in mutant SOD1 transgenic mice. Since therapies identified from studies of the mouse model have not so far translated to the clinic, the investigators propose to test the immunization strategy in a different ALS disease model. Canine degenerative myelopathy (DM) is an inherited adult-onset
neurodegenerative disease of dogs caused by mutations in the *SOD1* gene and thus is similar to amyotrophic lateral sclerosis (ALS), particularly familial forms that are associated with *SOD1* mutations. Investigators propose that this larger animal disease model may be beneficial in the preclinical development of the immunization strategy, as well as other drug therapies, and more accurately predict therapies that will halt or slow progression of ALS. This is a pilot study developing the feasibility of this larger model for testing clinical approaches to ALS.

**MILTON SAFENOWITZ POST DOCTORAL FELLOWSHIPS**

The Association also offers The Milton Safenowitz Postdoctoral Fellowship for ALS Research Award. Founded by the Safenowitz family through the Greater New York Chapter of The ALS Association and in memory of Mr. Safenowitz, who died of ALS in 1998, these awards are to encourage and facilitate promising young scientists to enter the ALS field.

**Researcher:** Russell McLaughlin, Ph.D., Trinity College, Dublin, Ireland

**Topic:** Use of Extended Irish Kindreds to Identify Novel ALS Variants

*Funded by The ALS Association, Greater Chicago Chapter, through the State of Illinois*

**Description:** ALS is a diverse disease with multiple causes. Despite having a clear heritable component, only 10 percent of ALS cases in Ireland can be explained by established genetic factors. This suggests that there are still many ALS genes to be discovered. As technological capabilities increase, whole-genome sequencing represents an exciting avenue in the discovery of novel disease genes. However, careful selection of informative individuals for whole-genome sequencing is paramount in experimental design. The Irish population is well-suited to genetic analysis, given its small size and the relative lack of mixture from other populations. Using existing datasets, Dr. McLaughlin and colleagues have developed methodologies to discover distant relationships amongst individuals previously assumed unrelated (e.g., third cousins). The proposed project will extend this to identify groups of patients from Ireland and the UK who are commonly descended from the same recent ancestor, thus identifying multiple affected members of extended families as ideal candidates for whole-genome sequencing. This challenges the distinction between sporadic and familial ALS and will help in the discovery of novel ALS genes. This is imperative for a better understanding of the underlying mechanism of the disease and will prove vital in patient classification in ALS research, drug development, disease management and clinical trials.

**Researcher:** Jonathan Labbadia, Ph.D., Northwestern University, Chicago, Illinois

**Topic:** Programmed re-modeling of the heat shock response underlies onset of ALS

*Funded by The ALS Association, Greater Chicago Chapter, through the State of Illinois*

**Description:** The causal relationship between aging and disease remains mysterious; however, an age-related change in the ability to prevent protein damage has been put forward as a trigger for the onset of neurodegeneration. If DNA is the "blueprint of life," then the proteins
it encodes are its effectors. Cells contain a huge number of proteins that are integral to cell function. If proteins become misfolded or mislocalized they can cause disease; therefore, maintaining protein integrity is essential for health. To achieve this, cells have evolved the protein homeostasis network (PN), a combination of pathways that suppress the presentation and persistence of aberrant proteins. As cells age, the PN becomes compromised, a phenomenon that is exacerbated in many age-related diseases, including ALS. Incredibly, aging studies in worms have revealed that a “switch-like” re-modeling of the PN is an early event in adulthood. Therefore, Dr. Labbadia and colleagues hypothesize that genetic “re-programming” of the PN underlies the increased susceptibility of individuals to protein misfolding and onset of ALS with age. They will test this hypothesis by identifying the molecular and genetic basis of PN re-modeling and use this knowledge to determine the impact of genetically re-engineering the PN on disease presentation in models of ALS.

**Researcher:** Yang Li, Ph.D., Barrow Neurological Institute, Phoenix, Arizona

**Topic:** Structure and function of a new candidate ALS biomarker RBM45 (RNA binding protein 45)

**Description:** Members of this lab have recently identified a novel RNA-binding protein RBM45 implicated in ALS and FTLD. RBM45 was initially identified through its altered levels in the cerebrospinal fluid of ALS patients. Further characterization indicates that RBM45 localizes to cytoplasmic inclusions and co-localizes with TDP-43 and ubiquitin inclusions in ALS and FTLD patients. Since joining the lab, Dr. Li has been focusing on how RBM45 is involved in the molecular pathway of neurodegeneration. She has discovered the physical interactions between RBM45 and other ALS-associated proteins and found that RBM45 can self-interact. She will continue to characterize the protein-protein interactions and the self-aggregation propensity of RBM45 in this study. She will also identify the RNA species that are bound by RBM45 by using a novel non-radioactive CLIPseq approach termed TAP-CLIP. TAP-CLIP can be applied to any RNA-binding protein to identify their RNA targets. This proposed study will provide novel insights into the function of RBM45 and its potential contribution to ALS pathogenesis.

**Researcher:** Jennifer Gass, Ph.D., Mayo Clinic, Jacksonville, Florida

**Topic:** Understanding the relationship between progranulin, sortilin1 and TDP43

*Funded by Jay & Toshiko Tompkins*

**Description:** Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the degeneration of motor neurons in the brain and spinal cord, which result in muscle weakness, atrophy and spasticity. A major pathological feature of ALS is the abnormal accumulation of a protein called TDP-43. In disease, TDP-43 is redistributed from the nucleus to the cytoplasm of a cell, truncated and abnormally phosphorylated. Mutations in the progranulin gene (GRN) resulting in decreased production of the progranulin protein are associated with TDP-43 pathology in frontotemporal lobar degeneration, a neurodegenerative disease sharing many clinical and pathological features with ALS. Recently, GRN mutations were also discovered in patients with ALS. Within the brain, progranulin levels are regulated by its
neuronal receptor, sortilin (SORT1). Currently there is no cure for ALS; however, therapeutic
treatments that inhibit the aberrant aggregation, mislocalization and truncation of TDP-43 may
prove beneficial for the treatment of ALS. The goal of this project is to determine whether
dysfunction with SORT1/progranulin exacerbates TDP-43 pathology and, ultimately, if
pharmacological progranulin enhancement alleviates TDP-43 pathology associated with ALS.
These studies will provide further insight into the mechanisms causing pathological TDP-43
accumulation and provide a therapeutic strategy for the treatment of ALS.

Researcher: Zhaoming Su, Ph.D., The Scripps Research Institute, La Jolla, California

Topic: Rational Design of Small Molecules Targeting GGGGCC Expanded Repeat in C9ORF72 gene implicated in ALS-FTD

Description: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are
incurable neurodegenerative diseases with a common genetic cause: an expanded repeat of the
sequence GGGGCC in the C9ORF72 gene. Like all genes, C9ORF72 is transcribed into RNA, which encodes proteins that complete most of the work in a cell. Once transcribed into RNA, the repeat causes ALS-associated defects. The researchers have previously developed a strategy to design drugs that target other expanded RNA repeats that cause disease. These designed drugs alleviated disease-associated defects in cellular and animal models. They will use similar design principles and strategies to develop drugs that bind the RNA expansion and improve ALS-associated defects. They have identified two lead drug candidates that bind the expansion in vivo and improve an ALS-associated defect. Based on these promising results, they propose to:

(i) optimize the two compounds by synthesizing and screening millions of derivatives for binding the expansion.
(ii) study the optimal compounds for alleviating two ALS-associated defects in patient-derived cell lines. The best compounds serve as potential therapeutics for ALS.
(iii) identify all biomolecules that the drugs bind in ALS patient-derived cells, thus determining drug mode of action and selectivity.

Researcher: Qiang Zhu, Ph.D., Ludwig Institute for Cancer Research, La Jolla, California

Topic: Determining the function of C9ORF72 gene and the pathogenic mechanisms of its hexanucleotide repeat expansion in amyotrophic lateral sclerosis

Description: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized clinically by progressive motor neuron degeneration, muscle wasting and paralysis. Currently there is no effective cure for this devastating motor neuron disease. Several important ALS-related mutants including SOD1, TDP-43 and FUS/TLS have been identified and studied extensively in the past years. Recently a novel large hexanucleotide repeat expansion in the non-coding region of the previously uncharacterized C9ORF72 gene has been discovered as the cause of the largest proportion of inherited ALS, which strongly links to ALS. Dr. Zhu will
exploit gene targeting and BAC transgenic approaches to establish mouse models with compromised C9ORF72 function and/or C9ORF72 hexanucleotide expansion as a means to explore pathogenic mechanisms (including loss of function and/or gain of RNA-mediated toxicity). Moreover, these mice will be utilized for testing efficacy of antisense oligonucleotide for targeted degradation of the C9ORF72 mRNA. Dr. Zhu’s hope is that these efforts will provide a greater understanding of disease mechanism and provide the rationale and basis for therapy development in ALS patients carrying C9ORF72 mutation.

**Researcher: Marka van Blitterswijk, M.D., Ph.D. Mayo Clinic, Jacksonville, Fla.**

* Funded by The ALS Association, Greater Chicago Chapter, through the State of Illinois

**Description: The discovery of chromosome 9 open reading frame 72 (C9ORF72) repeat expansions as the major genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) has changed the field of ALS research. Patients with these repeat expansions can present with many clinical signs and symptoms and have demonstrated huge variability in age at onset and disease duration. The research proposed in this grant aims at finding genetic variants that could contribute to these differences. The researchers plan on investigating 30 well-characterized patients with C9ORF72 expansions who have ALS and/or FTD and compare their pathological and clinical features to their entire genetic background. This comparison will enable us to identify potential disease modifiers, which will be of great predictive value for genetic counselors, and additionally, it could provide novel targets for the development of treatment strategies.**

For questions on these and other research grants, please email: researchgrants@alsa-national.org.